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NEW DITERPENOID

[Shinkina Diterupenoido]

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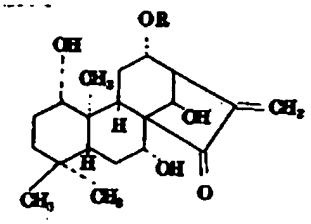
Specification

1. Title of the invention

NEW DITERPENOID

2. Claim

A diterpenoid, characterized by being represented by a general formula.

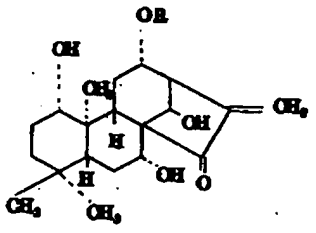


(In the formula, R represents a hydrogen atom or acetyl group.)

3. Detailed explanation of the invention

The present invention pertains to a new diterpenoid.

The diterpenoid of the present invention is represented by the following general formula (I).



(In the formula, R represents a hydrogen atom or acetyl group.)

The diterpenoid of the present invention represented by the above-mentioned general formula (I) has a carcinostatic action and is useful as medical supplies.

It has been known that Oridonin as a diterpenoid having a carcinostatic action exists in Isodon japonicus and Rabdosia rubesens which are Labiatae plants. These inventors succeeded in isolating two kinds of new diterpenoids represented by the /2 above-mentioned general formula (I) having an excellent carcinostatic action from Rabdosia exisa of the Labiatae plant different from these plants and completed the present invention.

The Rabdosia exisa being used in the manufacture of the compound of the present invention is a perennial herb being distributed in Unnam province, Wonneyong province, Sachon province, Hobuk province, etc., in China. Its stalks are rectangular and upright and reach about 50 cm-1 m, and its leaves are attached to its opposite stems. This plant has leaves with a shape whose tip is sharpened, such as egg shape or circular egg shape with a size of about 3.5-5.5 cm x 5-7 cm. It has been known that a boiled liquid of its leaf and stem parts has an antiphlogistic action, and it is internally used in oral

diseases and sore throat or used as an external drug in an arthralgia of hand and foot or neck parts. However, its components are not elucidated yet, and it is not known that a component having a carcinostatic action exists in said plant.

The compound of the present invention is isolated as follows from an ether extract in which the above-mentioned Rabdosia exisa is extracted with ether in advance according to an ordinary method. In other words, the above-mentioned extract is enriched, dried, dissolved in methanol, treated with an activated carbon, enriched, dried, and dissolved in acetone. The acetone solution is passed through a neutral alumina column and eluted with benzene and ether, so that a compound in which R of the above-mentioned general formula I is an acetyl group (hereinafter, this benzene extract is called "Exisanin B" and a compound in which R is a hydrogen atom (hereinafter, this ether eluate is called "Exisanin A") are respectively obtained. Details of the above-mentioned manufacturing method and the properties of the compounds of the present invention are clarified by application examples that will be mentioned later.

In using the compounds of the present invention being obtained in this manner as drugs, formulations corresponding to the dosage paths can be formed using ordinary formulation carriers. For example, for an oral dosage, the compounds are

prepared as tablets, capsules, granules, powders, liquid formulations, etc., and for a parenteral dosage, the compounds are prepared as injections, suppositories, etc. As the carriers that can be used in preparing the compounds as solid shapes for an oral dosage, ordinary excipients, binders, lubricants, colorants, disintegrators, etc., can be used. As the excipients, for example, lactose, sucrose, starch, talc, magnesium stearate, crystal cellulose, methyl cellulose, carboxymethyl cellulose, glycerin, sodium alginate, gum arabic, etc., can be used, and as the binders, polyvinyl alcohol, polyvinyl ether, ethyl cellulose, gum arabic, shellac, white sugar, etc., can be used. As the lubricants, magnesium stearate, talc, etc., can be used, and as the colorants and disintegrators, ordinary well-known substances can be used. Also, the tablets may be coated with a well-known method. Also, the liquid formulations may be aqueous or oily suspensions, solutions, syrups, elixirs and the like and are prepared by ordinary methods. In case the injection is prepared, pH adjustor, buffer agent, stabilizer, isotonic agent, local anesthetic, etc., are added to the compounds of the present invention, and hypodermic, intramuscular, and intravenous injections can be manufactured by ordinary methods. As base agents for manufacturing the suppositories, for example, oil and

fat base agents such as cacao butter, polyethylene glycol, lanolin, fatty triglyceride, and Uidepzol (trademark, Dynamite Nobel Co.) can be used. /3

The amount of dose of the formulations (carcinostatic agent) prepared in this manner depend on symptoms, weights, ages, etc., of patients and cannot be uniformly limited. However, the compounds of the present invention may be an amount in a range of usually about 50-1,000 mg a day for an adult and are preferably dosed by dividing them into 1-4 times a day.

Next, the manufacturing methods, the properties, and the pharmacological actions of the Exisanin A and the Exisanin B as the compounds of the present invention are explained in further detail by application examples.

Application Example 1

9 kg dried Rabdosia exisa was crushed and extracted with 20 L ether, so that a dark green ether extract was obtained. The ether extract was enriched and dried to attain 480 g coarse extract, and 5 L methanol was added to it and dissolved. Then, 400 g activated carbon was added to it, filtered, and decolored, so that a yellow methanol solution was recovered. The methanol solution was enriched, so that 98 g extract was obtained. 9.3 g of the extract was dissolved in 200 mL acetone and sent to a column of 200 g neutral alumina, and the initial 1,400 mL was

washed away by eluting with benzene (an elution rate of 3 mL/mm). Then, 1,200 mL of the benzene eluate was sampled and enriched, so that 2.6 g white powder Exisanin B with a melting point of 240-243°C was obtained.

$[\alpha]^{20}_{D}$ = -13.9 (C = 1.00, pyridine)

Element analysis value (as $C_{22}H_{32}O_6$)

Theoretical value (%): C 67.32, H 8.22

Actual measured value (%): C 67.75, H 8.24

ν_{max}(KBr): 3400, 1740, 1726, 1718, 1646, 1250, 1104, 1023, 990, 975, and 895 cm^{-1}

λ_{max}(C_2H_5OH): 230 nm (ε = 7901)

Application Example 2

The column of Application Example 1 was further eluted with ether (an elution rate of 3 mL/mm), and 800 mL of the ether eluate was sampled and enriched, so that 1.6 g white powder Exisanin A with a melting point of 262-264°C was obtained.

$[\alpha]^{20}_{D}$ = -27.7° (C = 1.01, pyridine)

Element analysis value (as $C_{20}H_{30}O_5$)

Theoretical value (%): C 68.54, H 8.63

Actual measured value (%): C 68.20, H 8.71

ν_{max}(KBr): 3430-3380, 1713, 1645, 1260, 1099, 1080, 1021, 1000, 968, and 938 cm^{-1}

λ_{max}(C_2H_5OH): 234 nm (ε = 5557)

<Pharmacological test>

The anitumor effects of the Exisanin A and the Exisanin B obtained in Application Examples 1 and 2 were tested using mouse-transplantable tumor P^{388} and sarcoma 180.

1 x 10^6 pieces of P^{388} cells/mouse were transplanted into the abdominal cavities of the BDF1 male mice (25-29 g), and 5 x 10^6 pieces of sarcoma 180 cells/mouse were transplanted into the abdominal cavities of ICR/JCL male mice (27-30 g).

The samples (Exisanin A or Exisanin B) were dissolved or suspended in a physiological saline solution and dosed into the abdominal cavities once a day for 7 days at a volume ratio of 1.0 mL/100 g weight of six mice as one group from the next day of the tumor transplantation. The amount being dosed was 2.5, 5, 10, or 20 mg/kg/day for each of the Exisanin A and the Exisanin B, and the number of average survival day each amount/4 of dose was attained. They were compared with the number of average survival day in an untreated control group to which only a physiological saline solution containing no compounds of the present invention was dosed, and the life extension increase rate (%) was calculated according to the following equation.

Life extension increase rate (%) = the number of average survival day of the sample dosed group - the number of average survival day of the untreated control group / the

number of average survival day of the untreated control group x 100

The results obtained are shown in the following Table I.

Table I

	P^{388}		Sarcoma 180	
Amount dosed (mg/kg/day)	Number of average survival day (day)	Life extension increase rate(%)	Number of average survival day (day)	Life extension increase rate(%)
Control group	9.86	-	13.75	-
Sample dosage group				
Exisanin A2.5	11.74	19	14.67	7
5	12.46	26	19.07	39
10	13.93	41	23.83	73
20	12.67	28	21.23	54
Exisanin B2.5	14.00	42	15.83	15
5	14.00	42	22.16	61
10	12.80	30	23.56	71
20	10.67	8	19.84	44